EXHIBIT S

Comparison of the *In Vivo* Behavior of Polyvinylidene Fluoride and Polypropylene Sutures Used in Vascular Surgery

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To find a nonabsorbable suture material that is equivalent to polypropylene in ease of handling and tensile properties, and that has low thrombogenicity and tissue reactivity but improved biostability, some researchers and clinicians see merit in considering the suitability of monofilaments made from polyvinylidene fluoride. The current animal study investigated the relative biocompatibility and biostability of these two suture materials by using them to anastomose a polyester arterial prosthesis in a canine thoracoabdominal bypass model for 10 periods of implantation ranging from 4 hr to 2 years. Biocompatibility was assessed with light and scanning electron microscope examinations of the explanted sutures, and biostability of the cleaned sutures was determined by Fourier transform infrared spectroscopy and scanning electron microscope analysis. The polyvinylidene fluoride and polypropylene sutures were found to have similar handling and healing characteristics. During the first months *in vivo*, both types of suture experienced a temporary increase in carbonyl group absorption that coincided with the duration of the inflammatory response. After 1 and 2 years *in vivo*, the explanted polypropylene sutures showed visual evidence of surface stress cracking. This was not found with the explanted polyvinylidene fluoride sutures. These results suggest that polyvinylidene fluoride may be more biostable than polypropylene in the long term. *ASAIO Journal* 1998; 44:199–206.

Sutures are the most frequently implanted biomaterial in humans, and are used widely in all fields of surgery.[1,2] The subject of suture performance, however, has received little attention by the surgical community, because reported failures have been mainly anecdotal.[3–5] Ideally, a suture should be easy to manipulate, have superior elastic and mechanical properties, exhibit low thrombogenicity and tissue reactivity, and provide both chemical and physical biostability over time. Polypropylene was introduced as a suture material in the late 1950s.[2] It has high flexibility and tensile strength, and exhibits low thrombogenicity and tissue reaction. It has proved resistant to infection, but appears vulnerable to iatrogenic trauma.

In recent years, however, the safety and efficacy of using polypropylene sutures in various surgical procedures has been questioned.[6–16] Degradation and failure phenomena, such as surface fragmentation and fracture at a location distant from the knot, have been observed in both vascular and ophthalmologic surgery. Therefore, there is interest by the medical device industry in seeking new alternative biomaterials, and in this context, the potential use of polyvinylidene fluoride (PVDF) as a suture material is under consideration.[17] Preliminary studies have shown recently that PVDF sutures have similar breaking strength, higher extension at break, good physicochemical characteristics, and superior tensile creep resistance to polypropylene sutures.[17,18] In addition, it has been found that they are less susceptible to iatrogenic trauma than polypropylene sutures.[18]

The purpose of the current study was to investigate the relative *in vivo* biocompatibility and biostability of these new PVDF monofilament sutures compared with polypropylene controls. Of particular interest was their ability to heal and remain chemically stable *in vivo* when anastomosed to a polyester arterial prosthesis implanted as a canine thoracoabdominal bypass for periods ranging between 4 hr and 2 years. The healing behavior of the PVDF and polypropylene sutures was measured in terms of their tissue reaction using light and scanning electron microscopy. After cleaning to remove adhering tissue, their relative biostability was assessed in terms of surface morphology and chemistry using scanning electron microscopy and Fourier transform infrared spectroscopy.

Materials and Methods

Materials

The PVDF monofilament suture used in this study was developed by Péters Laboratoire Pharmaceutique (Bobigny, France), and made commercially available in Europe under the trade name Teflene®. The control suture was a polypropylene monofilament manufactured by Ethicon (Johnson and Johnson, Peterborough, Ontario, Canada) under the trade name Prolene® **(Figure 1).**

In Vivo *Implantation*

Animal selection and hematologic tests. Twenty adult female mongrel dogs, weighing between 16 and 20 kg, were selected and housed according to the Canadian Council on Animal Care regulations. In addition to routine hematologic tests (*i.e.,* hematocrit, platelet, and leukocyte counts), the blood sample taken from each dog was characterized by platelet aggregation analysis and thromboelastography.

Surgery and animal follow-up. The thoracoabdominal by-

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Figure 1. Scanning electron photomicrographs of virgin 5−0 polyvinylidene fluoride (A, ×100) and polypropylene (B, ×100) sutures.

pass model, first described by McCune and Blades,[19] and frequently used at our institution for the evaluation of new grafts, was used in the current investigation. Implantations were performed for 10 prescheduled periods of 4, 24, and 48 hr; 1 and 2 weeks; 1, 3, and 6 months; and 1 and 2 years using a gelatin sealed polyester arterial prosthesis. Two dogs were given implants for each period. Before surgery, each dog was fasted for 24 hr, then anesthetized with 32 mg/kg intravenous sodium pentobarbital (Somnotol®; MTC Pharmaceutical Ltd., Mississauga, Ontario, Canada), intubated, and mechanically ventilated. In addition, 20 mg/kg of Ketamine hydrochloride (Rogar STB Inc., Montreal, Quebec, Canada) was administered to maintain anesthesia as required. Intravenous infusions of Ringer's lactate were injected to compensate for dehydration during surgery. The abdomen and the thorax were shaved, and the skin was prepared with 4% chlorhexidine gluconate (Ayerst, Montreal, Quebec, Canada) and with a 10% proviodine topical solution (Rougier, Chambly, Quebec, Canada). The thoracic aorta was isolated by means of a left thoracotomy through the eighth intercostal space. The abdominal aorta was mobilized from the renal arteries to the aortic trifurcation through a midline lower abdominal incision.

A polyester arterial prosthesis, manufactured by Vascutek (Inchinnan, Scotland) and measuring 8 mm in diameter and 30 cm in length, was implanted in each dog. The animals were given 0.5 mg/kg heparin intravenously (Allen and Hanburys, Glaxo Canada Ltd., Toronto, Ontario, Canada) at least 5 min

before vascular clamping. The distal anastomosis was performed in an end to side manner between the prosthesis and the infrarenal aorta, using a 6−0 suture. The graft was then tunnelled in the retroperitoneal space, and the proximal end to end anastomosis was completed with a 5−0 suture. Two series of implantations were performed, 10 dogs in each; one series used PVDF monofilament sutures, and the other polypropylene monofilament sutures as the control. The abdomen and thorax were closed in layers using 1−0 and 2−0 polypropylene sutures. The animals were returned to their cages and fed an unrestricted standard diet. All animals received 3 ml penicillin antibiotic (Rogar STB Inc.) intramuscularly at the time of anesthesia.

Graft Harvesting

The dogs were returned to the operating room and anesthetized with 32 mg/kg of sodium pentobarbital intravenously. Blood samples were taken for hematologic analysis. Heparin (0.5 mg/kg) was administered intravenously to minimize post mortem thrombotic deposits over the graft surface. The dogs were exsanguinated *via* the right femoral artery. The grafts were removed by a thoracophrenolaparatomy, opened longitudinally, and carefully rinsed. The two anastomotic sites were then photographed with a Tessovar microphotography optical system (Carl Zeiss, Oberkochen, Germany) to assess the macroscopic findings. Proximal and distal anastomoses were cut in two, one for histologic investigation, and the other for morphologic and chemical studies after cleaning.

Histologic Study

Histologic examinations were conducted on the PVDF and polypropylene sutures removed from both anastomotic sites after each had been divided into two specimens, one for light and the other for scanning electron microscopy. One sample was fixed in a 2% isotonic buffered glutaraldehyde solution, rinsed in distilled water, and post fixed in osmium tetroxide. Drying was completed by immersion in a series of ethanol solutions of increasing concentration, followed by critical point drying using liquid carbon dioxide as the transfer medium. The specimens were then coated with gold palladium and examined in a Jeol JSM 35CF (Soquelec, Ltd., Montreal, Quebec, Canada) scanning electron microscope at a 15 kV accelerating voltage. The second sample was fixed in a 10% solution of formalin and embedded in paraffin, and 5 μm thick sections were cut and stained with hematoxylin, eosin, Weigert, and Masson's trichrome.

Morphologic and Chemical Studies

Cleaning procedure. The second half retrieved from each anastomotic site was cleaned by removing all adherent tissue with an enzyme incubation technique developed in our laboratory.[20] Special care was taken when handling the sutures during this cleaning process. The specimens were first cleaned with 500 U/ml of collagenase Type 1A (Sigma, St. Louis, MO) in a 50 mmol/L Tris-HCl buffer at pH 7.5 containing 0.36 mmol/L of $CaCl_2$ for 24 hr at 37°C. The specimens were then incubated in a 0.5% solution of pancreatin in 25 mmol/L Tris-HCl buffer at pH 7.5 for 24 hr at 37°C. After several washes in deionized water, the specimens were soaked in 1% Triton X-

Table 1. Summary of the Macroscopic and Microscopic Observations at the Surface of the PVDF Sutures After Implantation in a Canine Thoracoabdominal Bypass Model

Duration of Implantation	Proximal	Distal
4 hours	Small thrombotic deposits	Small thrombotic deposits
24 hours	Clean surface	Small thrombotic deposits
48 hours	Small thrombotic deposits	Clean surface
1 week	Collagen and thrombotic matrix	Collagen and thrombotic matrix
2 weeks	Thin collagen covering	Thin collagen covering, macrophages, lymphocytes
1 month	Collagen, few macrophages and giant cells	Little collagen, macrophages
3 months	Collagen, absence of inflammatory cells	Collagen, few macrophages
6 months	Collagen, few macrophages	Collagen, absence of inflammatory cells
1 year	Collagen, absence of inflammatory cells	Collagen, absence of inflammatory cells
2 years	Collagen, absence of inflammatory cells	Collagen, fibroblasts, few macrophages

100 non-ionic surfactant (Sigma) for 24 hr at room temperature, then rinsed in deionized water for 24 hr, and vacuum dried at 40°C under a pressure of 30 mmHg for $\geq$48 hr. The sutures were then gently removed from the polyester arterial prostheses with a plastic tweezer. Virgin PVDF and polypropylene sutures of 5–0 and 6–0 sizes also were cleaned using the same procedure to distinguish between those changes that resulted from implantation from those, if any, that were due to the cleaning procedure. The surfaces of the cleaned and control sutures were inspected in the scanning electron microscope to assess any surface modifications.

Fourier transform infrared spectroscopy. Infrared spectra were recorded with a Nicolet Magna-550 (Nicolet Instruments Corp., Radison, WI) Fourier transform infrared spectrometer with a deuterated triglycine sulfate detector and a germanium coated potassium bromide beam splitter. Five hundred scans were taken with an optical retardation of 0.25 cm. They were then triangularly apodized and Fourier transformed to yield a 4 cm^{-1} resolution. Because of their thickness ($\approx$100–200 μm), the sutures were opaque to infrared radiation. Therefore, the attenuated total reflectance mode was used to obtain the infrared spectra near the surface. A split pea attachment (Harrick Scientific Corp., Ossining, NY), equipped with a silicon hemispheric internal reflection element 3 mm in diameter and bevelled on the edge of its flat surface, provided a fixed sampling area slightly larger than the 150–200 μm diameter hot spot on the crystal, and ensured that all specimens were mounted under the same pressure. The spectral baselines between 1,000 and 2,000 cm^{-1} were connected using a straight line.

The level of absorbance was measured at the 1,740 cm^{-1} infrared band. This peak has been assigned to carbonyl stretch-

ing, and identifies the presence of surface oxidation, because the chemical structures of both pure polymers are devoid of this functional group.[18] The use of GRAMS software (Galactic Industries Corp., Salem, NH) facilitated the calculation of total absorbance at this wave number.

Results

Surgery and Animal Follow-Up

All operations were successful without morbidity or mortality. Both types of sutures exhibited satisfactory and equivalent handling and suturing characteristics. The suturing time required to complete both anastomoses in the two series of implantations was indistinguishable between the PVDF and polypropylene sutures. The mean $\pm$ standard deviation of these times were 31.5 $\pm$ 4.4 and 30.5 $\pm$ 3.8 min, respectively. Bleeding through both anastomoses after restoration of the blood flow within the vascular prostheses was minimal for both types of sutures, and when it did occur, it was easily controlled within minutes.

Macroscopic and Microscopic Observations

The vascular grafts explanted after different times of implantation were all patent. Macroscopic examination of the luminal surface of the polyester grafts after the first 24 hr of implantation revealed a few small thrombotic deposits. Thereafter, at 48 hr and 1 week, a thin regular thrombotic matrix was found covering the entire graft. After 1 month, the flow surface was in the process of reorganization, having a smooth and glistening appearance near both anastomoses, and a few isolated parietal

Table 2. Summary of the Macroscopic and Microscopic Observations at the Surface of the Polypropylene Sutures After Implantation in a Canine Thoracoabdominal Bypass Model

Duration of Implantation	Proximal	Distal
4 hours	Fibrin, platelets	Fibrin, platelets
24 hours	Clean surface	Clean surface
48 hours	Fibrin, platelets	Small thrombotic deposits
1 week	Thin collagen covering, fibroblasts	Covered by thrombotic matrix
2 weeks	Small thrombotic deposits	Covered by thrombotic matrix, erythrocytes, macrophages
1 month	Thin collagen covering, fibroblasts	Thin collagen covering, fibroblasts
3 months	Collagen, absence of inflammatory cells	Collagen, fibroblasts
6 months	Collagen, absence of inflammatory cells	Collagen, absence of inflammatory cells
1 year	Collagen, absence of inflammatory cells	Collagen, absence of inflammatory cells
2 years	Collagen, absence of inflammatory cells	Collagen, absence of inflammatory cells

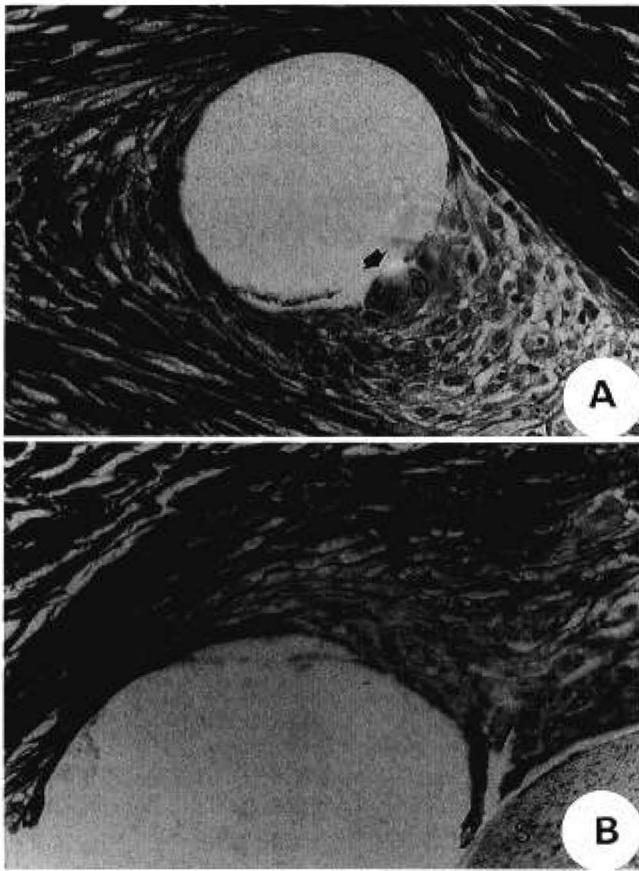202   MARY *ET AL.*

Figure 2. Light photomicrographs of polyvinylidene fluoride sutures at the distal anastomotic site after 1 and 3 months of implantation. At 1 month, the suture is surrounded by fibroblast-like cells, collagen deposits, and a few macrophages (arrow) (A, ×500). After 3 months, the presence of inflammatory cells around the sutures (S) was rarely observed (B, ×500).

thrombi in the medial region. After longer implantation periods of 1 and 2 years, the luminal surface of the grafts was completely covered by a collagenous capsule, without any evidence of thrombotic deposits.

Summaries of the macroscopic and microscopic observations of the surfaces of the explanted PVDF and polypropylene sutures are presented in **Table 1** and **Table 2,** respectively. Both types of sutures experienced a similar healing sequence characterized by the deposition of a small amount of thrombotic debris on the surface of the monofilaments during the first week of implantation. After this time, the PVDF sutures started to become encapsulated by newly formed collagen, and after 1 month were fully covered by a thin collagenous lining. The polypropylene sutures had a similar healing response, although the formation of a continuous collagen lining appeared to be delayed. Microscopic observations of the anastomoses after periods between 3 months and 2 years revealed that both sutures were encapsulated in thicker collagenous tissue without any invasion by inflammatory cells **(Figures 2–5).**

Surface Morphology

After implantation times of up to 6 months, it was found that the cleaned surfaces of both PVDF and polypropylene sutures

were well preserved, without any evidence of surface pitting or cracking. Evidence of iatrogenic trauma was occasionally observed, however, with both types of sutures. This phenomenon is inevitable, and results from the manipulation of the monofilaments with the tweezer or needle holder during the anastomotic procedure. After 1 and 2 years of implantation, the surface of the retrieved and cleaned PVDF sutures did not appear to be substantially modified **(Figure 6).** In contrast, the polypropylene sutures explanted 1 and 2 years postoperatively showed evidence of surface deterioration, characterized by uniformly spaced circumferential cracking and peeling and flaking of the polymer material in the outermost surface layer **(Figure 7).**

Fourier Transform Infrared Spectroscopy

Compared with standard reference infrared spectra, the observed Fourier transform infrared spectroscopy spectra exhibited the typical peaks one expects to find with these two polymers.[21] In addition, the virgin and retrieved and cleaned samples of both polymers gave a weak absorption at $1,740$ cm^{-1},
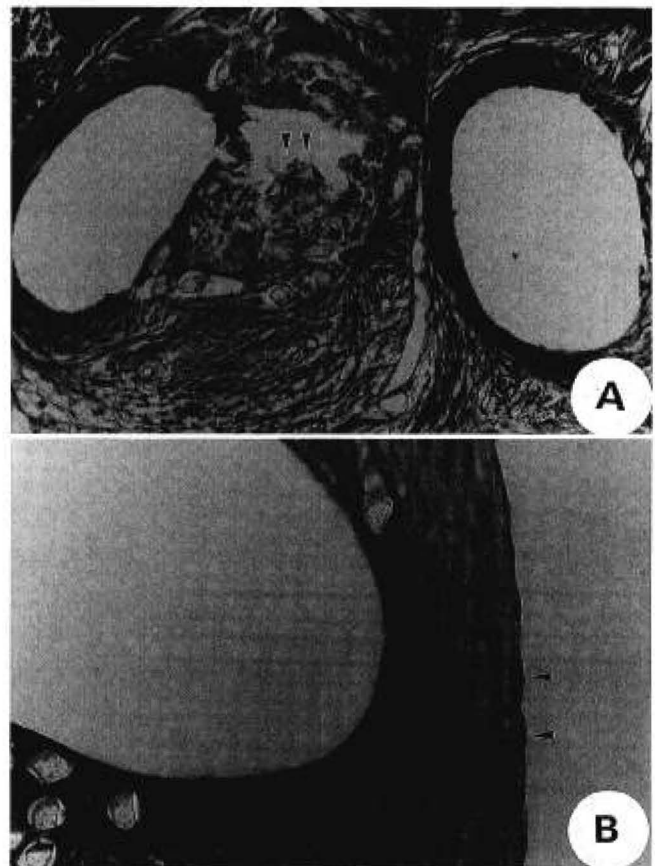

Figure 3. Light photomicrographs of polyvinylidene fluoride sutures explanted at 6 months. The sutures located adjacent to the prosthesis were subjected to a chronic inflammatory response directed toward the polyester fibers (arrows). Nevertheless, they were covered by a thin lining of collagenous tissue (A, ×250). Near the luminal surface, the suture was encapsulated by the newly formed internal collagenous capsule without any inflammatory cells. Endothelial-like cells can be observed covering the collagen tissue (arrows) (B, ×500).
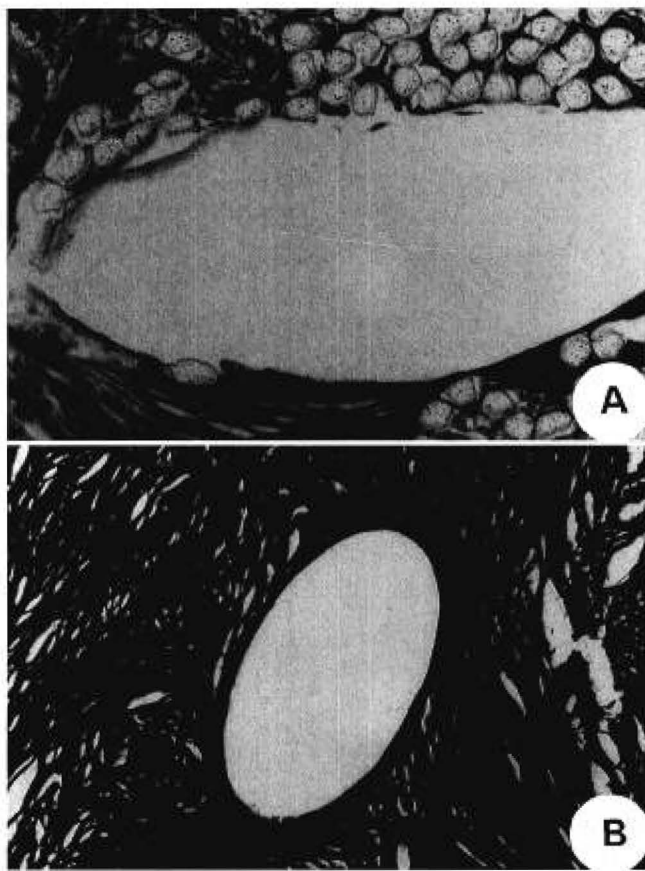
Figure 4. Light photomicrographs of polyvinylidene fluoride sutures after implantation for 1 and 2 years. At 1 year, the suture located in the graft wall was encapsulated by collagen infiltrating the polyester skeleton without evidence of an inflammatory reaction (A, ×500). Sutures examined after 2 years also revealed complete encapsulation by collagenous tissue without the presence of inflammatory cells (B, ×500).

which has been assigned to the carbonyl group and gives information about the extent of surface oxidation. The results for the two materials have been plotted vs the implantation time in **Figure 8.**

It is noteworthy that all four curves have some similar characteristics. The PVDF and polypropylene sutures experienced an early increase in carbonyl absorbance soon after implantation, which reached a maximum value within the first 1–3 months. After this time, it decreased to an average value, where it remained approximately constant for at least 1 year postoperatively. The timing of the peak seems to differ for the two types of suture—at 1 month for PVDF and 3 months for polypropylene **(Figure 8).** This period corresponds to the formation of a continuous collagenous capsule around the sutures **(Tables 1, 2),** and the difference in timing may be explained by localized differences in the rates of healing between the two types of suture.

Discussion

For many years, polypropylene has been recognized as an inert suture material because of its excellent biostability and mechanical properties. Its molecular weight was believed to

be too high for the polymer to be readily broken down by enzymatic degradation and used as an energy source by microorganisms.[22] Several studies have demonstrated that polypropylene monofilament sutures induce minimal loss of tensile strength and low tissue response in vivo.[1,2] For these reasons, polypropylene has become the monofilament suture of choice for peripheral vascular surgery. There is evidence to suggest, however, that polypropylene monofilament sutures are sensitive to iatrogenic trauma.[18,23] Anecdotal reports also reveal that suture fracture may occur in ophthalmic[6,7] and vascular surgery.[8,24] It has long been known that polypropylene is susceptible to degradation by several different initiation phenomena including thermal, mechanical, photochemical, radiation, biologic, and chemical mechanisms.[25]

In recent in vitro and in vivo studies, we have examined the physicochemical properties, ease of handling, and biocompatibility of a new vascular suture made from PVDF. Compared with polypropylene, the PVDF monofilament suture showed better long-term stability in vitro by retaining 92.2% of the initial tensile strength over a 9 year period, whereas polypropylene retained only 53.4%.[21] The PVDF suture also exhibited minimal cellular and tissue reactions in vivo.[17,18,21]
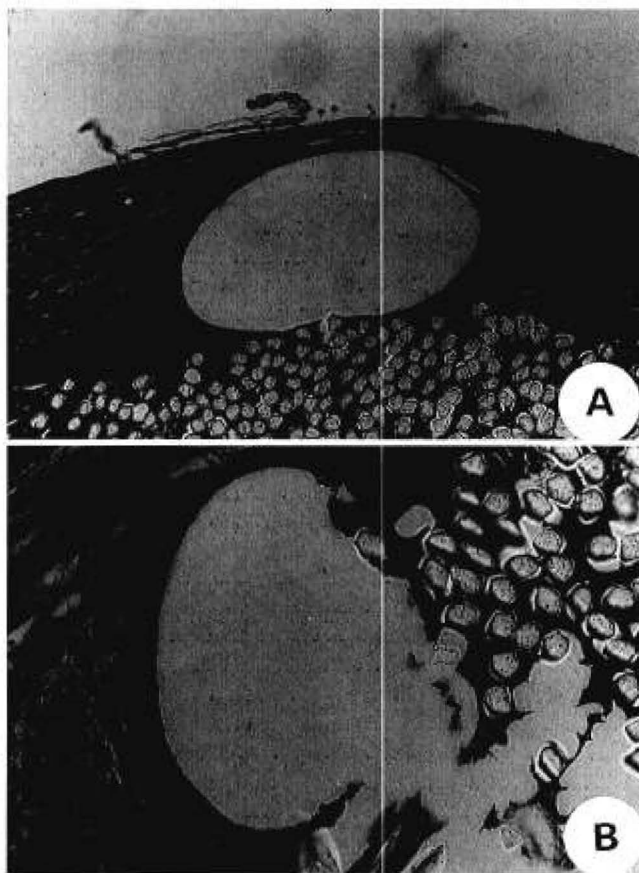

Figure 5. Light photomicrographs of polypropylene sutures retrieved from the anastomotic sites of vascular grafts after 3 and 6 months implantation. After 6 months, the sutures located in the collagenous tissue covering the luminal surface of the graft do not exhibit any inflammatory response (A, ×250). The sutures present in the polyester grafts are surrounded by fibroblast-like cells and collagenous tissue without the infiltration of inflammatory cells (B, ×500).
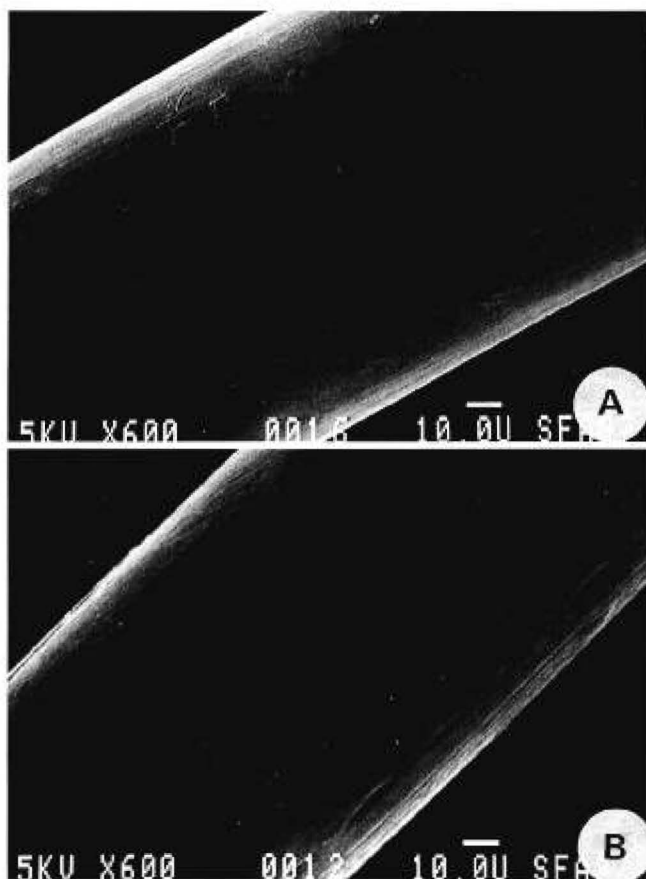
Figure 6. Scanning electron photomicrographs of retrieved and cleaned 6−0 polyvinylidene fluoride sutures showing the surface morphology after implantation for 1 (A, ×600) and 2 (B, ×600) years *in vivo.*
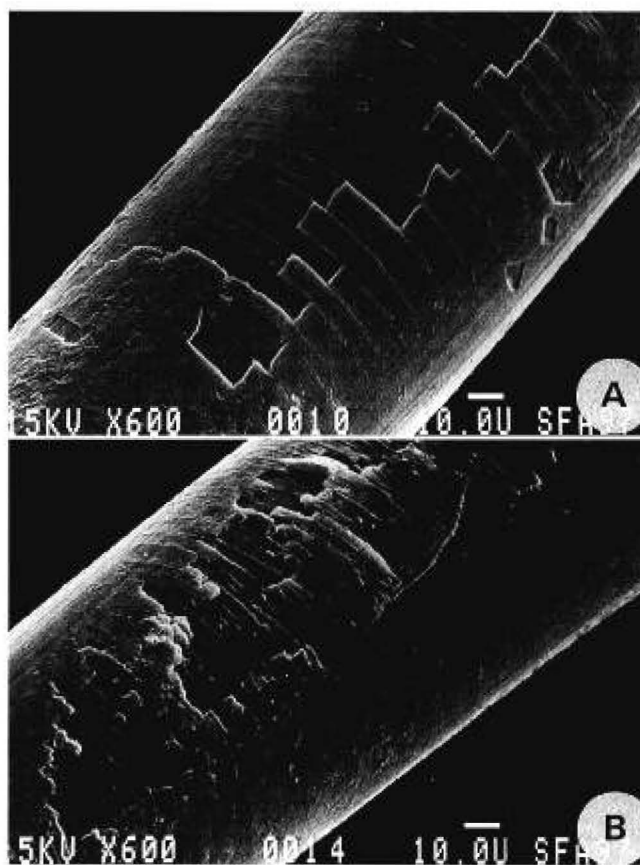

Figure 7. Scanning electron photomicrographs of retrieved and cleaned 6−0 polypropylene sutures showing the surface morphology after implantation for 1 (A, ×100) and 2 (B, ×600) years *in vivo.*

The current investigation was undertaken to compare the long-term chemical and physical stability, biocompatibility, and tissue response of a PVDF monofilament suture with those of a typical polypropylene suture. Our study confirmed that both PVDF and polypropylene virgin sutures contain some background level of oxidative species, even before implantation. The reason for this is not clearly understood, but is possibly due to a predisposition of the material to oxidation during certain manufacturing and sterilization processes. For example, the thermal treatments during spinning and swaging can result in the formation of hydroperoxides in the outer layers of polypropylene monofilaments.[25] Traces of transition metals added as components of the polypropylene polymerization reaction can act as catalysts to decompose hydroperoxides and peroxides to active free radicals, even in the presence of antioxidants.[25] Alternatively, the sterilization conditions using ethylene oxide or radiation are known to generate oxidized species in the surface layers of polypropylene sutures.[26]

The reason for the observed increase in carbonyl content over the first 1–3 months in the current study may be due to the generation of free radicals and peroxides by the oxidative burst and inflammatory cellular response, which are then converted to hydroperoxides and carbonyl residues.[27] This phenomenon has been demonstrated previously by Liebert *et al.,* who identified an oxidative process, chain scission, and the

formation of carbonyl groups within a few days of implanting polypropylene sutures subcutaneously in rats.[27] Ultimately, this process led to a loss in breaking extension and tensile strength and embrittlement of the sample. This hypothesis is supported by the finding that the observed surface carbonyl
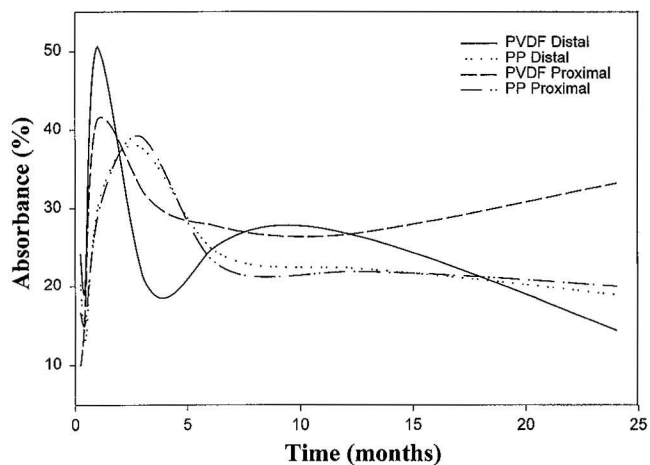

Figure 8. Carbonyl absorbance of polyvinylidene fluoride and polypropylene sutures after different implantation times.

content of both PVDF and polypropylene sutures decreased to the initial background level once a continuous collagenous capsule had formed and actively metabolizing inflammatory cells were no longer present.

The phenomenon of surface oxidation is only one of a series of steps in the degradation process. After formation of free radicals and chain scission, degradation of polypropylene monofilaments involves surface embrittlement and crack formation and the loss of mechanical properties.[28] In this study, the scanning electron microscopy images of the polypropylene sutures after 1 and 2 years *in vivo* showed evidence of typical circumferential stress cracking, with the cracks spaced uniformly at intervals of 3–5 μm along the filament axis **(Figure 7)**. This phenomenon has been observed on other occasions when the degradation process has been initiated by a variety of different mechanisms.[6,7,29–32]

A scanning electron microscopy study of polypropylene sutures explanted from the human eye has shown similar evidence of slow surface degradation, as was found in this study. Sutures exposed to actively metabolizing tissue exhibited linear and circumferential surface cracking and flaking after 2 years of implantation.[6] Greenwald *et al.* have demonstrated that, after 6 weeks of implantation in the rat, non-absorbable polypropylene sutures experience significant losses in mechanical properties, particularly in lower breaking strain, tensile strength, and toughness.[31]

The reason for this stress cracking phenomenon in oriented polypropylene monofilaments has been explained by their pronounced skin/core structure. This bicomponent structure is created by the differential cooling rates between the external and internal layers of the monofilaments during the melt spinning process, which leads to the formation of a low order nonfibrillar outer skin a few microns thick, and a highly oriented crystalline fibrillar inner core.[33] Blais *et al.* identified a distinct separation and different properties between these two layers. They found that the outer skin is more susceptible to oxidative degradation than the fibrillar inner core. Cleavage of the polymer chains causes relaxation of the folded lamellae, increases in crystallinity and density, and contraction localized to the outer skin. This in turn leads to regular circumferential crack formation at the surface, but only to the depth of the outer layer.[33] Because this cracking is confined to the outer skin, which is clearly distinguishable from the inner core structure, it is not surprising to observe that, during abrasive stresses, such as cleaning, there was a tendency for the cracked rings at the surface to flake off and separate from the underlying core material **(Figure 7).**

The reason why this cracking phenomenon was not observed with the explanted PVDF sutures after 1 year and 2 years *in vivo* is not known at this time. One possible explanation for the apparent superior biostability of the PVDF monofilaments, however, may lie in their less pronounced bicomponent skin/core structure, which is attributable to their different manufacturing history.[17]

Because the level of oxidation products, either hyperoxide or carbonyl groups, near the surface of polypropylene monofilament sutures has been found to be an unreliable index of the extent of deterioration in mechanical properties, additional *in vitro* fatigue testing is required.[33,34] By exposing the sutures to controlled enzymatic, oxidative, acidic, alkaline, and radiation conditions over the long term, it may be possible to de-velop a model that will clarify our understanding of the relevant degradation mechanisms.

Conclusions

The findings from this preliminary long-term animal trial point to a number of conclusions. First, regarding ease of manipulation, the PVDF and polypropylene sutures have been found to have equivalent handling and knotting characteristics. Second, both types of sutures experience a similar healing sequence, with short-term deposition of fibrin, platelets, and thrombi giving way to the growth of a surrounding collagen capsule between 1 and 3 months postoperatively. Third, during the same period, surface oxidation was detected with both polymers. The growth in surface carbonyl content during the first 1–3 months coincides with the inflammatory phase, and then appears to decrease once a continuous collagenous capsule has developed. Visual evidence of surface degradation was observed after 1 and 2 years for the polypropylene but not the PVDF sutures. This stress cracking phenomenon is believed to be associated with the distinct skin/core two phase structure of oriented polypropylene monofilaments, and points to the likelihood of PVDF having superior biostability to polypropylene over the long term.

Acknowledgments

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